PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 38/27

A1

(11) International Publication Number: **WO 97/38709**

(43) International Publication Date: 23 October 1997 (23.10.97)

(21) International Application Number: PCT/SE97/00601

(22) International Filing Date: 10 April 1997 (10.04.97)

(30) Priority Data:
9601397-4 12 April 1996 (12.04.96) SE

(71) Applicant *(for all designated States except US)*: PHARMACIA & UPJOHN AB [SE/SE]; S-112 87 Stockholm (SE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: JOHANNSSON, Gudmundur [IS/SE]; Sahlgrenska University Hospital, Research Center for Endocrinology and Metabolism, S-413 45 Göteborg (SE). MÅRIN, Per [SE/SE]; Sahlgrenska University Hospital, Dept. of Heart and Lung Diseases, S-413 45 Göteborg (SE). LÖNN, Lars [SE/SE]; Sahlgrenska University Hospital, Dept. of Radiology, S-413 45 Göteborg (SE). OTTOSSON, Malin [SE/SE]; Sahlgrenska University Hospital, Dept. of Heart and Lung Diseases, S-413 45 Göteborg (SE). STENLÖF, Kaj [SE/SE]; Sahlgrenska University Hospital, Dept. of Medicine, S-413 45 Göteborg (SE). BJÖRNTORP, Per [SE/SE]; Sahlgrenska University Hospital, Dept. of Heart and Lung Diseases, S-413 45 Göteborg (SE). SJÖSTRÖM, Lars [SE/SE]; Sahlgrenska University Hospital, Dept. of Heart and Lung Diseases, S-413 45 Göteborg (SE). BENGTSSON, Bengt-Åke [SE/SE]; Sahlgrenska University Hospital, Research Center for Endocrinology and Metabolism, S-413 45 Göteborg (SE).

(74) Agents: TANNERFELDT, Agneta et al.; Pharmacia & Upjohn AB, S-112 87 Stockholm (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: USE OF GROWTH HORMONE

(57) Abstract

The invention relates to the use of growth hormone, preferably human growth hormone or analogues thereof for the manufacturing of a medicament for treatment of individuals with the Metabolic syndrome. They include individuals with abdominal/visceral obesity and its metabolic and circulatory consequences including insulin resistance, lipoprotein aberrations and hypertension. The medicament is also used to increase insulin sensitivity and for treatment and prevention of non-insulin dependent diabetes mellitus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

USE OF GROWTH HORMONE

The present invention relates to the use of growth hormone, preferably human growth hormone or analogues thereof for the manufacturing of a medicament for treatment of individuals with the Metabolic syndrome (also labeled Syndrome X). They include individuals with abdominal/visceral obesity and its metabolic and circulatory consequences including insulin resistance, lipoprotein aberrations and hypertension. The medicament is also used to increase insulin sensitivity and for treatment and prevention of non-insulin dependent diabetes mellitus.

Introduction

Striking similarities exhibits between The Metabolic Syndrome [1, 2] (also labeled Syndrome X or Primary Insulin Resistance Syndrome) and untreated GH deficiency in adults [3]. The most central findings in both these syndromes are abdominal/visceral obesity and insulin resistance [1, 4-6]. Other features common to both conditions are high triglyceride and low high-density lipoprotein cholesterol concentrations, an increased prevalence of hypertension, elevated levels of plasma fibrinogen and plasminogen activator inhibitor-1 activity, premature atherosclerosis and increased mortality from cardiovascular diseases [1, 4, 7-11].

The Metabolic Syndrome is associated with multiple endocrine abnormalities. They include increased cortisol secretion, blunted secretion of gonadotrophins and sex steroids and abnormalities in the GH/insulin-like growth factor-I (IGF-I) axis [12-14]. With increased adiposity GH secretion is blunted with decreased mass of

GH secreted per burst but without any major impact on GH secretory burst frequency [15]. The serum IGF-I concentration is principally GH dependent and influences GH secretion though a negative feed-back system [16]. The serum levels of IGF-I are inversely related to the percentage body fat [15]. In addition, we have previously shown that the low serum IGF-I concentration in obesity is predominantly related to the amount of visceral adipose tissue and not to the amount of subcutaneous fat mass [13]. These findings, together with other endocrine disturbances in central obesity, suggest that the low GH secretion which is observed is secondary to a central disturbance of the neuroendocrine regulation, including the GH/IGF-I axis.

The abdominal/visceral obesity and insulin resistance observed in The Metabolic Syndrome constitute the base for hypertension, dyslipoproteinemia and non-insulin dependent diabetes mellitus. [1]

Replacement therapy with recombinant human GH (rhGH) has demonstrated favorable effects on most of the features of GH deficiency in adults [17]. Whether rhGH treatment can improve the metabolic abnormalities observed in abdominal/visceral obesity has never been investigated. In the present study a randomized, double-blind, placebo-controlled design was used to evaluate the effects of rhGH administration in patients with abdominal/visceral obesity

In the present study thirty men, 48 to 66 years of age with abdominal/visceral obesity were treated with recombinant human GH (rhGH) in a 9-month randomized, double-blind, placebo-controlled trial. Body fat was assessed from total body potassium and abdominal subcutaneous and visceral adipose tissue was measured using computed tomography. Glucose disposal rate (GDR) was measured during euglycemic, hyperinsulinemic glucose clamp.

In response to the rhGH treatment, total body fat, abdominal subcutaneous and visceral adipose tissue decreased. The GDR (glucose disposal rate) increased in the rhGH-treated group as compared with the placebo-treated one. The mean serum concentrations of total cholesterol and triglyceride decreased while blood glucose and serum insulin concentrations were unaffected by the rhGH treatment. Furthermore, diastolic blood pressure decreased and systolic blood pressure was unchanged in response to rhGH treatment.

The present study has demonstrated that GH can favorably affect some of the multiple perturbations associated with The Metabolic Syndrome. This includes a reduction in abdominal/visceral obesity an improved insulin sensitivity and favorable effects on lipoprotein metabolism and diastolic blood pressure. Thus, the prevention and treatment of non-insulin dependent diabetes mellitus may be possible.

The findings must be regarded as unexpected and surprising and of utmost interest giving a possibility to treat and prevent diseases associated with increased cardiovascular morbidity and mortality.

INVENTION

The invention relates to the use of growth hormone, preferably human growth hormone or analogues thereof for the manufacturing of a medicament for treatment of individuals with the Metabolic syndrome (also labeled Syndrome X) as claimed in the claims on file.

By analog is meant a substance having the same biological activity as described here and having at least 65 % homology with natural occurring growth hormone.

Legends to figures.

Figure 1. Mean total body fat calculated from total body potassium, abdominal subcutaneous adipose tissue (AT) area at the level of L4-L5 and volume of visceral AT.

Figure 2. Abdominal subcutaneous and visceral adipose tissue determined with computed tomography at the level of L4-5 in one man before (A) and after 9 months of rhGH treatment (B).

Figure 3. Mean fasting blood glucose, serum insulin and glucose disappearance rate (GDR).

EXAMPLE

Subjects and methods

Patients

Thirty men (48 to 66 years of age) were studied (Table 1). They were recruited by advertisement in a local newspaper. The criteria for inclusion in the study were age approximately 50 to 65 years with a body mass index between 25 and 35 kg/m^2, an IGF-I less that 160 µg/L (low normal) [18] and a waist hip ratio of more that 0.95. The criteria for exclusion were overt diabetes mellitus, previous cardiovascular event or heart disease.

In the rhGH treated group, two patients were receiving treatment for hypertension with both atenolol (100 mg per day) and nifedipin (40 mg per day) and one patient had slight asthma, treated with salmeterol and terbutalin inhalations. In the placebo treated group, one patient had a slight depressive disorder and was receiving paroxetin (10 mg per day). These medications were kept stable during the study period.

Study design

This study was a 9 month randomized, double-blind, placebo-controlled trial of the administration of rhGH. Informed consent was obtained from each patient before the study. The study was approved by the Ethics Committee at the University of Göteborg and by the Swedish Medical Products Agency, Uppsala, Sweden.

Treatment

The daily rhGH dose was 9.5 µg/kg (0.20 IU/kg body weight/week), administrated subcutaneously before bedtime. The dose was reduced by half in the event of side-effects. The average dose reduction during the 9-month study was 0.17 mg per day (range -1.7 to 0). The placebo vials contained the same vehicle as the rhGH vials and both preparations were visually indistinguishable. Compliance assessed by counting the returned empty vials and expressing that number as a percentage of vials needed for the treatment period was 87.3 % (range 32-100).

Study protocol

The patients were studied as out-patients before and after 6 weeks, 6 and 9 months of treatment. Physical and laboratory examinations were performed at all visits. The euglycemic hyperinsulinemic clamp, adipose tissue needle aspiration and measurement of blood pressure and heart rate were performed at baseline, 6 weeks and after 9 months of treatment. Body weight was measured in the morning to the nearest 0.1 kg wearing indoor clothing, and body height was measured barefoot to the nearest 0.01 m. The body mass index was calculated as body weight in kilograms divided by height in meters squared. Waist circumference was measured in the standing position with a flexible plastic tape midway between the lower rib margin and the iliac crest, and the hip girth at the widest part of the hip.

Systolic and diastolic blood pressure were measured after 5 minutes of supine rest using the sphygmanometric cuff method. This measurement was repeated after one minute and the mean value was used.

Total-body potassium was measured by counting the emission of 1.46 MeV gamma-radiation from the naturally occurring ^{40}K isotope in a high-sensitive 3π whole body counter with a coefficient of variation (CV) of 2.2%. Fat-free mass (FFM) was estimated by assuming a potassium content of 64.7 mmol/kg FFM [19] and body fat (BF) was calculated as body weight-FFM. A five-scan computed tomography technique was used (Philips Tomoscan 350) to measure abdominal adipose tissue. The five levels were derived from two scanograms and included one scan at the level of the mid-thigh with the lower edge of symphysis as a reference point. The other four levels were the lower edge of symphysis, L4-5 lumbar disc, L3-4 lumbar disc and a scan at the level of liver and spleen. The tissue areas and anatomic boundaries were determined as described previously [20]. Total volume of visceral adipose tissue was determined from the five-scan model. Sagittal diameter and abdominal subcutaneous and visceral adipose tissue areas were determined at the level of L4-5.

A euglycemic, hyperinsulinaemic glucose clamp was performed after an overnight fast as previously described [14]. In brief, insulin was infused together with glucose into a venous catheter with the tip at the level of the axillary vein at appropriate rates to obtain submaximal insulin concentrations. Blood glucose concentrations were monitored every 10 min. and glucose infusion rate was adjusted to fasting levels. Glucose disappearance rate (GDR) was measured for 20 minutes in steady-state conditions, which was reached after 100 min. The insulin concentrations during steady state were 214 ± 10 μIU/mL before treatment, 226 ± 12 μIU/mL at 6 weeks and 213 ± 11 μIU/mL at 9 months. During the clamp, in steady-state conditions, a subcutaneous abdominal adipose tissue biopsy was obtained by

needle aspiration for the determination of lipoprotein lipase (LPL) activity. The needle aspiration was performed under local anesthesia, 0.1 m lateral to the umbilicus. The biopsies were immediately frozen in liquid nitrogen and stored at -80-C until assay.

Analytic methods

Total LPL activity in adipose tissue was determined after homogenization of the tissue in the detergent-containing buffer as described previously [21]. Bovine skim milk was used as a standard to correct for interassay variation. The amount of triglycerides (TG) in the tissue was measured after extraction [22] and weighed after evaporation of solvents. Activity was expressed in milliunits (1mU=1 mmol FFA released per min.) per gram adipose tissue and per gram TG. Control experiments showed that the assay was linear with the amount of sample and incubation time over the range used. The within-assay CV was 4.3%.

Blood samples were drawn in the morning after an overnight fast. The serum concentration of insulin-like growth factor-I (IGF-I) was determined by a hydrochloric acid-ethanol extraction radioimmunoassay using authentic IGF-I for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with within-assay CV of 2.5% and 4.2% at serum concentrations of 125µg/L and 345µg/L respectively. The standard deviation score for IGF-I was then calculated from predicted IGF-I values adjusted for age and sex obtained from the normal population [18].

The insulin-like growth factor binding protein-3 (IGFBP-3) concentration in serum was determined by radioimmunoassay (Nichols Institute Diagnostics) with total CV of 6.2% and 5.7% at serum concentrations of 2.05 mg/L and 3.49 mg/L respectively.

Serum cholesterol and triglyceride concentrations were determined with enzymatic methods (Boehringer, Mannheim, Germany). The within-assay CV for total cholesterol and triglyceride determinations was 0.9% and 1.1% respectively.

Fibrinogen was measured according to a syneresis method [23] with a total CV of 4% at 2.5 g/L. Plasminogen activator inhibitor (PAI)-1 activity was measured using a Spectrolyse (pL) PAI kit (Biopool Stabilyte, Umeå, Sweden) with a total CV of 10% at concentrations between 10-40 IU/ml.

Serum insulin was determined by a radioimmunoassay (Phadebas, Pharmacia, Uppsala, Sweden) and blood glucose was measured by the glucose-6-phosphate dehydrogenase method (Kebo Lab, Stockholm, Sweden). Hemoglobin A1c (HbA1c) was determined by high pressure liquid chromatography (Waters, Millipore AB, Sweden) and C-peptide was determined by a radioimmunoassay (Santec, USA). Free fatty acids (FFA) levels were determined using an enzymatic colorimetric method (NEFAC; Wako, Neuss, Germany), while sex hormone binding globulin (SHBG) was measured by an immunoradiometric assay (Farmos, Diagnostica, Åbo, Finland) and total testosterone by a radioimmunoassay (ICM, Biomedical, Costa Mesa, USA).

Statistical methods

All descriptive statistical results are presented as the means and standard error of the mean (SEM). Comparisons between the two treatment groups were performed by a two-way analysis of variance (ANOVA) for repeated measurements. Comparisons between baseline values and values at 9 months in the two groups were performed with the Students' t-test for independent groups. Correlations were sought by calculating the Pearson linear correlation coefficient. Pearson's chi square test was used to test the independence of frequency of hypertension and smoking between the rhGH and placebo treated groups. Before statistical analysis.

logaritmic transformation of data with skewed distribution was performed. A two-tailed probability value of less than 0.05 was considered significant.

Results

The two groups were matched with regard to age, body height, body weight and waist hip ratio, and did not differ significantly regarding number of subjects with medically treated hypertension (Chi sqr.=1.87; p=0.17) and current smokers (Chi sqr.=1.2; p=0.27) (Table 1). At baseline the two groups did not differ significantly from each other in any of the variables studied.

Body composition

The patients' mean overall body mass index and fat-free mass did not change while mean total body fat decreased by 9.2 ± 2.4% during rhGH treatment in comparison with placebo treatment (Figure 1). Waist circumference and sagittal diameter decreased in response to rhGH while no change occurred in the placebo group. Moreover, abdominal subcutaneous and visceral adipose tissue area at the level of L4-5 decreased in response to rhGH by 6.1 ± 3.2% and 18.1 ± 7.6% respectively (Figure 1 and Table 2). The corresponding values in the placebo group were +2.0 ± 2.8% and -3.2 ± 7.6% respectively. Moreover, the volume of visceral adipose tissue decreased in the rhGH treated group by 17.9 ± 3.5% while no change was observed in the placebo treated group (-0.2±4.2%) (Figure 1).

Thus, the percentage of visceral adipose tissue of the total adipose tissue at the level of L4-5 decreased by 14.5 ± 3.8% ,while the percentage of subcutaneous adipose tissue of the total adipose tissue at the level of L4-5 increased by 5.4 ± 1.7 % in the rhGH-treated group.

Abdominal subcutaneous and visceral adipose tissue was determined with computed tomography at the level of L4-5 in one man before (A) and after 9

months of rhGH treatment (B). The scan showed a reduction of both visceral and subcutaneous adipose tissue . (Figure 2)

Glucose metabolism

No significant effects were elicited by rhGH treatment on blood glucose, serum insulin and HbA1c (Table 3 and Figure 3). The serum concentration of C-peptide increased in the rhGH treated group due to a transient increase at 6 weeks, but the C-peptide level was similar in the two groups at 9 month.

In the rhGH treated group the GDR showed an initial decrease after 6 weeks of treatment followed by an increment while the placebo-treated group demonstrated a slight reduction over time (Figure 3). The average increase in GDR in response to 9 months of rhGH was 1.2 ± 0.7 mg/kg/min compared with baseline while in the group receiving placebo a slight decrease by 0.4 ± 0.6 mg/kg/min occurred. This difference between the two groups was still found after correcting the GDR for the amount of fat-free mass.

In the rhGH treated group, an inverse correlation was found between the change in GDR and change in serum triglyceride concentration ($r=-0.58$; $p<0.05$) but no significant correlations were found between the change in GDR and changes in serum IGF-I concentration ($r=-0.24$), serum insulin concentration ($r=-0.27$), LPL activity ($r=0.09$), diastolic blood pressure ($r=-0.30$), total body fat ($r=-0.06$) and volume of visceral adipose tissue ($r=-0.11$).

Total cholesterol, triglycerides, LPL activity, plasma fibrinogen and PAI-1 activity (Table 3)

The mean total cholesterol concentration decreased from 6.1 ± 0.2 to 5.4 ± 0.3 mmol/L and the triglyceride concentration decreased from 2.09 ± 0.29 to 1.78 ± 0.23 mmol/L in response to rhGH treatment. The corresponding changes in the

placebo group were from 5.4 ± 0.3 to 5.5 ± 0.2 mmol/L and from 1.65 ± 0.13 to 2.05 ± 0.26 mmol/L respectively.

The mean total LPL activity in subcutaneous abdominal tissue did not change during rhGH treatment in comparison with placebo treatment. However, between 6 weeks and 9 months, LPL activity tended to increase in the rhGH treated group as compared with the placebo treated group ($p=0.06$). Similar results were obtained when LPL activity was expressed in mU/g TG.

The plasma concentration of fibrinogen increased in response to rhGH while PAI-I activity was unaffected by rhGH treatment as compared with placebo.

Blood pressure and heart rate

Diastolic blood pressure decreased from 75 ± 2 to 70 ± 2 mmHg in the rhGH treated group. The corresponding values in the placebo group were from 73 ± 2 to 74 ± 2 mmHg ($p<0.05$). No significant effects on systolic blood pressure or heart rate were observed between the two treatment groups.

Serum IGF-I, IGFBP-3, testosterone and SHBG (Table 4)

Before treatment, the serum IGF-I concentration was low normal in both treatment groups. A significant increment in serum IGF-I and IGFBP-3 concentrations occurred in the rhGH treated group as compared with placebo treatment. At 6 weeks, the serum IGF-I concentration reached on average 3.30 ± 0.35 SD above the predicted mean in the rhGH treated group while at 9 month the mean serum IGF-I concentration was 1.89 ± 0.48 SD above the predicted mean. Serum concentrations of testosterone and SHBG were not significantly affected by rhGH treatment.

Side-effects

No drop-outs occurred during the study. Side-effects were observed in 8 subjects in the rhGH treated group, and were mainly a result of fluid retention. Five had peripheral edema, two subjects experienced muscle stiffness and arthralgia, one developed mild carpal tunnel syndrome and one subject experienced increased perspiration. These side-effects appeared during the first 6 weeks of treatment and subsided in four patients in response to a reduction in dose performed within 4 months after start of treatment; in three patients, the side-effects subsided spontaneously. One man from the rhGH treated group with medically treated hypertension was taken off treatment after 8 months when he experienced an intracerebral hemorrhage. Three subjects in the placebo group experienced slight and transient peripheral edema.

Legends to figures.

Figure 1.

Mean total body fat calculated from total body potassium, abdominal subcutaneous adipose tissue (AT) area at the level of L4-L5 and volume of visceral AT assessed with computerized tomography during 9 months of treatment with rhGH or placebo in 30 men with abdominal/visceral obesity. The horizontal bars indicate the SE for the mean values shown and p-values denotes the differences between the two groups by two-way ANOVA for repeated measurements.

Figure 2.

Abdominal subcutaneous and visceral adipose tissue determined with computed tomography at the level of L4-5 in one man before (A) and after 9 months of rhGH treatment (B). The scan shows the reduction of both visceral and subcutaneous adipose tissue (shown as dark gray area in the figure).

Figure 3.

Mean fasting blood glucose, serum insulin and glucose disappearance rate (GDR) assessed with euglycaemic hyperinsulinaemic glucose clamp during 9 months of treatment with rhGH or placebo in 30 men with abdominal/visceral obesity. The horizontal bars indicate the SE for the mean values shown and p-values denotes the differences between the two groups by two-way ANOVA for repeated measurements.

Discussion

We have shown that 9 months of rhGH treatment in middle-aged men with abdominal/visceral obesity reduced total body fat and resulted in a specific and marked decrease of both abdominal subcutaneous and visceral adipose tissue. Insulin sensitivity assessed with euglycemic glucose clamp technique improved and serum concentrations of total cholesterol and triglyceride decreased. Furthermore, diastolic blood pressure decreased.

The men who were studied were moderately obese with a preponderance of abdominal and/or visceral localization of body fat as judged from comparisons with randomly selected men of comparable age from the same city [24]. As a group, they had slight to moderate metabolic changes known to be associated with abdominal/visceral obesity with moderate insulin resistant as judged from the GDR values obtained during the euglycemic glucose clamp, although no one had overt diabetes.

Although, we used a lower daily rhGH dose than previously reported in trials studying healthy adults [25, 26], the initial rhGH doses administrated were apparently too high as judged by the frequency of side-effects and initial high average serum IGF-I concentrations. After dose reduction the average serum IGF-I concentration where within the normal range indicating that the doses of rhGH

during the latter part of the study were more physiological. This might in turn explain the less marked anabolic action of the rhGH treatment demonstrated in this study in comparison with previous trials in healthy adults [25, 26].

The marked effect of GH replacement on body composition in GH-deficient adults has been a consistent observation in many studies [17]. The profound lipolytic effect of GH was also demonstrated in the present study with a preferential reduction in visceral adipose tissue depots [27]. These changes have been associated with a GH-induced reduction in the antilipolytic effects of insulin, which is markedly different in different adipose tissue regions [28].

GH exerts direct insulin-antagonistic effects even after the administration of physiological doses of rhGH. GH has been considered to be the principal factor in the decrease in insulin sensitivity observed in the early morning, the so called "dawn phenomenon" [29] and the insulin resistance following hypoglycemia [30]. Thus, our observation of increased insulin sensitivity during prolonged rhGH treatment is unexpected. We have previously shown that 6 weeks of rhGH treatment in GH-deficient adults only induced a temporary decrease in insulin sensitivity which after 6 months of treatment was restored to baseline values [31]. The response of GDR to rhGH treatment on in this trial showed a similar initial tendency to induce insulin resistance but after 9 months a marked improvement was found.

GH has been found to be an important regulator of the hepatic LDL-receptor [35] and the overall lipoprotein metabolism [36]. The reduction in total cholesterol is conceivable an effect of enhanced hepatic LDL receptor activity in response to GH[35]. In healthy adults, short-term rhGH administration has been reported to increase serum triglyceride concentration [25]. In this study, the serum triglyceride concentration also displayed an initial increase in response to rhGH treatment. This could be an effect of both an increased flux of FFA to the liver and a direct

stimulatory effect on the esterification of oleic acid into triglyceride and phospholipids in hepatocytes [37] in response to GH which in turn enhances the very low density lipoprotein production from the liver. However, after 9 months of rhGH treatment serum triglyceride concentration had decreased again, probably as an effect of the increased insulin-stimulated glucose uptake which is known to be inversely related with the very low density lipoprotein secretion rate from the liver and serum triglyceride levels [4].

The initial tendency of a decline in LPL activity observed in the present study is in accordance with a previous 2 weeks treatment trials with rhGH [38]. During the more prolonged rhGH treatment, however, LPL activity tended to increase which may, at least in part, explain the decrease in serum triglyceride concentration at 9 months. The changes in GDR and LPL activity showed a similar biphasic pattern in response to GH. It may thus be speculated that the suggested influence of GH on LPL activity is mediated through insulin sensitivity, since insulin is known to be a potent stimulator of LPL.

Nine months of rhGH treatment reduced diastolic blood pressure without affecting systolic blood pressure. This is in line with results from GH-deficient adults where rhGH administration reduced diastolic blood pressure possibly as an effect of reduced peripheral vascular resistance [39]. The mechanisms behind the reduction in peripheral vascular resistance might be indirect through the reduced abdominal obesity and increased insulin sensitivity [40] or more direct through the action of IGF-I on the vascular wall [41].

Abdominal/visceral obesity is associated with increased plasma fibrinogen concentration and PAI-1 activity [42, 43] which both are established risk factors for myocardial infarction and stroke [44, 45]. The slightly increased plasma fibrinogen concentration in response to GH may be mediated through increased serum IGF-I concentration [18]. In GH-deficient adults, 2 years of rhGH treatment tended to

decrease plasma fibrinogen levels and diminish PAI-1 activity [46]. This further illustrates the importance of the duration of rhGH treatment on the metabolic effects of GH.

Previous studies have shown that testosterone treatment of middle-aged men with abdominal/visceral obesity induced improved insulin sensitivity, plasma lipid levels, diastolic blood pressure as well as a specific decrease of visceral adipose tissue mass [14, 46]. Since testosterone treatment of men with hypogonadotrophic hypogonadism increases GH secretion [47] the similarities between testosterone and rhGH treatment might be explained by increased GH levels or by additive or synergistic effects of GH and testosterone on adipose tissue metabolism [48].

The multiple endocrine alterations associated with abdominal/visceral obesity can either be primary responsible or be the consequence of the obese condition. This is the first trial clearly to demonstrate favorable effects of GH on the multiple perturbations associated with abdominal/visceral obesity. We therefore suggest that a blunted GH secretion could be an important factor in the development of the metabolic and circulatory consequences of abdominal/visceral obesity. The metabolic effects demonstrated in this study are probably of importance for the risk of cardiovascular morbidity and mortality in men with abdominal and central adiposity.

Table 1.

Clinical characteristics of the cohort of 30 men treated with GH or placebo for 9 months.

Patient characteristics	GH	Placebo
Number of men	16	14
Mean age (range); years	58.3 (48-66)	57.9 (52-63)
Treated hypertension; no.	2	0
Present smokers; no.	5	2
Body height; m	1.80±0.02 *	1.77±0.02
Body weight; kg	101.7±2.4	96.2±2.6
Waist hip ratio	1.01±0.01	1.03±0.02

* Plus-minus values are means ± standard error of the mean.

Table 2.

Measurements of body mass index (BMI), fat free mass, waist circumference, sagittal diameter and abdominal visceral adipose tissue (AT) area at the level of L4-5 in 30 men, during 9 months of rhGH or placebo treatment.

Variable		Baseline	6 weeks	6 months	9 months	p #
BMI; kg/m²						
	GH	31.4±0.7	31.6±0.7	31.3±0.7	31.1±0.8	0.2
	Placebo	30.5±0.8	30.6±0.7	30.5±0.8	30.7±0.8	
Fat free mass; kg						
	GH	67.5±1.8	69.7±1.9	71.1±1.8	69.5±2.2	0.4
	Placebo	64.6±1.4	65.2±1.4	66.4±1.3	65.3±1.4	
Waist; cm						
	GH	111.8±1.8	110.8±1.8	107.6±1.7	109.8±1.9	0.002
	Placebo	109.5±2.5	109.4±2.4	109.3±2.3	111.0±2.3	
Sagittal diameter;cm						
	GH	26.1±0.5	25.9±0.5	25.2±0.5	25.0±0.6	0.03
	Placebo	25.5±0.7	25.3±0.8	24.6±0.9	25.5±0.8	
Visceral AT; cm²						
	GH	126±15	121±19	98±14	99±15	0.004
	Placebo	163±16	147±13	142±12	150±13	

All values are expressed as the mean (SEM).

P-values denote the differences between the two groups by two-way ANOVA for repeated measurements.

Table 3.

Measurements of glycosylated hemoglobin (HbA1c), C-peptide, total cholesterol, triglycerides, lipoprotein lipase (LPL) activity expressed as mU per gram of adipose tissue (AT), plasma fibrinogen and plasminogen activator inhibitor (PAI)-1 in 30 men, during 9 months of rhGH or placebo treatment.

Variable	Baseline	6 weeks	6 months	9 months	p #
HbA1c; %					
GH	5.2±0.1	5.2±0.1	5.4±0.1	5.3±0.1	0.8
Placebo	4.9±0.1	4.9±0.1	5.0±0.1	5.1±0.2	
C-peptide; ng/L					
GH	1.11±0.10	1.60±0.13	1.30±0.17	1.18±0.07	0.02
Placebo	1.28±0.27	1.30±0.27	1.28±0.32	1.42±0.30	
Total cholesterol; mmol/L					
GH	6.1±0.2	5.7±0.2	6.1±0.2	5.4±0.3	0.006
Placebo	5.4±0.3	5.6±0.2	6.1±0.3	5.5±0.2	
Triglycerides; mmol/L					
GH	2.09±0.29	2.60±0.42	2.31±0.23	1.78±0.23	0.02
Placebo	1.65±0.13	1.80±0.20	1.85±0.22	2.05±0.26	
LPL activity; mU/g AT					
GH	287±26	205±15	ND	371±65	0.2
Placebo	224±31	228±29	ND	263±25	
Fibrinogen; g/L					
GH	3.13±0.13	3.44±0.10	3.49±0.13	3.24±0.19	0.04
Placebo	3.03±0.11	2.73±0.09	3.39±0.28	2.84±0.10	
PAI-1 activity; U/mL					
GH	31.3±6.4	23.2±2.6	18.3±3.1	22.9±3.5	0.4
Placebo	17.0±3.8	22.9±4.2	19.9±4.5	27.1±6.3	

All values are expressed as the mean (SEM).

P-values denote the differences between the two groups by two-way ANOVA for repeated measurements.

Table 4.

Measurements of serum IGF-I, IGFBP-3, free fatty acids (FFA), testosterone and steroid hormone binding globulin (SHBG) in 30 men, during 9 months of GH/placebo treatment.

Variable		Baseline	6 weeks	6 months	9 months	p #
IGF-I; µg/L						
	GH	134±8	338±16	320±23	268±23	<0.001
	Placebo	120±11	121±11	129±12	119±12	
IGF-I SD score *						
	GH	-0.82±0.17	3.30±0.35	2.91±0.49	1.89±0.48	<0.001
	Placebo	-1.07±0.23	-1.04±0.23	-0.87±0.24	-1.08±0.25	
IGFBP-3; mg/L						
	GH	2.36±0.13	3.20±0.10	3.19±0.13	2.71±0.14	0.001
	Placebo	2.10±0.16	2.21±0.17	2.45±0.21	2.18±0.19	
FFA; µmol/L						
	GH	0.77±0.07	0.99±0.11	0.78±0.11	0.75±0.07	0.5
	Placebo	0.73±0.05	0.73±0.05	0.59±0.06	0.67±0.05	
Testosterone; nmol/L						
	GH	14.6±1.2	12.9±1.0	13.9±1.3	12.4±1.1	0.12
	Placebo	14.6±1.0	15.4±1.2	17.6±2.1	14.6±1.3	
SHBG; nmol/L						
	GH	26.4±3.4	25.5±3.5	25.9±3.1	23.4±2.8	0.8
	Placebo	28.1±2.9	29.1±3.1	29.2±3.2	25.5±2.7	

All values are expressed as the mean (SEM).

P-values denotes the differences between the two groups by two-way ANOVA for repeated measurements.

* The standard deviation score for serum IGF-I is calculated from predicted IGF-I values adjusted for age and sex obtained from the normal population [18].

References

1. Björntorp P. Visceral obesity: A "Civilization syndrome". Obes Res 1993;1; 206-222.

2. Reaven GH. Role of insulin resistance in human disease. Diabetes 1988;37:1595-1607.

3. Bengtsson B-Å. The consequences of growth hormone deficiency in adults. Acta Endocrinol 1993;128(Suppl 2):2-5.

4. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75:473-486.

5. Salomon F, Cuneo RC, Hesp R, Sönksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 1989;321:1797-1803.

6. Johansson J-O, Fowelin J, Landin K, Lager I, Bengtsson B-Å. Growth hormone-deficient adults are insulin-resistant. Metabolism 1995;44:1126-9.

7. Rosén T, Edén S, Larsson G, Wilhelmsen L, Bengtsson B-Å. Cardiovascular risk factors in adult patients with growth hormone deficiency. Acta Endocrinol 1993;129:195-200.

8. Johansson J-O, Landin K, Tengborn L, Rosén T, Bengtsson B-Å. High fibrinogen and plasminogen activator inhibitor activity in growth hormone-deficient adults. Arterioscler Thromb 1994;14:434-7.

9. Markussis V, Beshyam SA, Fisher C, Sharp P, Nicolaides AN, Johnston DG. Detection of premature atherosclerosis by high-resolution ultrasonography in symptom-free hypopituitary adults. Lancet 1992;340:1188-92.

10. Mårin P, Björntorp P. Endocrine-metabolic pattern and adipose tissue distribution. Horm Res 1993;39(suppl 3):81-85.

11. Rosén T, Bengtsson B-Å. Premature mortality due to cardiovascular diseases in hypopituitarism. Lancet 1990;336:285-88.

12. Mårin P, Darin N, Amemiya T, Andersson B, Jern S, Björntorp P. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism 1992;41:882-886.

13. Mårin P, Kvist H, Lindstedt G, Sjöström L, Björntorp P. Low concentrations of insulin-like growth factor-I in abdominal obesity. Int J Obesity 1993;17:83-89.

14. Mårin P, Holmäng S, Gustafsson C, et al. Androgen treatment of abdominally obese men. Obes Res 1993;1:245-251.

15. Veldhuis JD, Liem AY, South S, et al. Differential impact of age, sex steroid hormones, and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J Clin Endocrinol Metab 1995;80:3209-22.

16. Hartman ML, Clayton PE, Johnson ML, et al. A low dose euglycemic infusion of recombinant human insilin-like growth factor I rapitly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. J Clin Invest 1993;91:2453-2462.

17. De Boer H, Blok G-J, Van der Veen EA. Clinical aspects of growth hormone deficiency in adults. Endocr Rev 1995;16:63-86.

18. Landin-Wilhelmsen K, Wilhelmsen L, Lappas G, et al. Serum insulin-like growth factor I in a random population sample of men and women:relationship to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin Endocrinol 1994;41:351-7.

19. Kvist H, Chowdhury B, Sjöström L, Tylén U, Cederblad Å. Adipose tissue volume determination in males by computed tomography and ^{40}K. Int J Obesity 1988;12:249-266.

20. Chowdhury B, Sjöström L, Alpsten M, Konstanty J, Kvist H, Löfgren R. A multicompartment body composition technique based on computed tomography. Int J Obesity 1994;18:219-234.

21. Bengtsson-Olivecrona G, Olivecrona T. Lipoprotein analysis-a practical approach. Oxford: Oxford University Press, 1992. (Converse CA, Skinner ER, eds. Practical Approach Series;

22. Dole VP, Meinertz H. Microdetermination of long-chain fatty acids in plasma and tissues. J Biol Chem 1960;235:2595-2599.

23. Friberger P, Knös M, Gustavsson S, Aurell L, Claeson G. Methods for determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. Heamostasis 1978;7:138-145.

24. Larsson B, Seidell J, Svärdsudd K, et al. Obesity, adipose tissue distribution and health in men. The study of men born in 1913. Apetite 1989;13:37-44.

25. Marcus R, Butterfield G, Holloway L, et al. Effects of short-term administration of recombinant human growth hormone to elderly people. J Clin Endocrinol Metab 1990;70:519-27.

26. Rudman D, Feller AG, Nagraj HS, et al. Effects of human growth hormone in men over 60 years old. N Engl J Med 1990;323:1-6.

27. Bengtsson B-Å, Edén S, Lönn L, et al. Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. J Clin Endocrinol Metab 1993;76:309-317.

28. Rosenbaum M, Gertner JM, Leibel R. Effects of systemic growth hormone (GH) administration on regional adipose tissue distribution and metabolism in GH-deficient children. J Clin Endocrinol Metab 1989;69:1274-81.

29. Bolli GB, Gerich JE. The "dawn phenomenon"-A common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. N Engl J Med 1984;310:746-750.

30. Fowelin J, Attvall S, von Schenk H, Smith U, Lager I. Combined effect of growth hormone and cortisol on late posthypoglycaemic insulin resistance in humans. Diabetes 1989;38:1357-1364.

31. Fowelin J, Attvall S, Lager I, Bengtsson B-Å. Effects of treatment with recombinant human growth hormone on insulin sensitivity and glucose metabolism in adults with growth hormone deficiency. Metabolism 1993;42:1443-47.

32. DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent diabetes mellitus. J Clin Invest 1985;76:149-155.

33. Lund S, Flyvbjerg A, Holman GD, Larsen FS, Pedersen O, Schmitz O. Comparative effects of IGF-I and insulin on the glucose transporter system in rat muscle. Am J Physiol 1994;267:E461-E466.

34. Ayling CM, Moreland BH, Zanelli JM, Schulster D. Human growth hormne treatment of hypophysectomized rats increases the proportion of type-1 fibres in skeletal muscle. J Endocrinol 1989;123:429-435.

35. Rudling M, Norstedt G, Olivecrona H, Reihnér E, Gustafsson J-Å, Angelin B. Importance of growth hormone for the induction of hepatic low density lipoprotein receptors. Proc Natl Acad Sci USA 1992;89:6983-7.

36. Angelin B, Rudling M. Growth hormone and hepatic lipoprotein metabolism. Curr Opin Lipidol 1994;5:160-5.

37. Elam MB, Wilcox HG, Salomon SS, Heimberg M. In vivo growth hormone treatment stimulates secretion of very low density lipoproteins by the perfused rat liver. Endocrinology 1992;131:2717-22.

38. Richelsen B, Pedersen SB, BÒrglum JD, MÒller-Pedersen T, JÒrgensen J, JÒrgensen JO. Growth hormone treatment of obese women for 5wk; effect on body composition and adipose tissue LPL activity. Am J Physiol 1994;266:E211-E216.

39. Caidahl K, Edén S, Bengtsson B-Å. Cardiovascular and renal effects of growth hormone. Clin Endocrinol 1994;40:393-400.

40. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities-the role of insulin resistance and the sympathoadrenal system. N Engl J Med 1996;334:374-381.

41. Copeland KC, Nair KS. Recombinant human insulin-like growth factor-I increases forearm blood flow. J Clin Endocrinol Metab 1994;79:230-2.

42. Vague P, Juhan-Vague I, Aillaud MF, et al. Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 1986;35:250-253.

43. Landin K, Stigendal L, Eriksson E, et al. Abdominal obesity is associated with impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. Metabolism 1990;39:1044-1048.

44. Hamsten A, de Faire U, Walldius G, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 1987;2:3-9.

45. Wilhelmsen L, Svärdsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med 1984;311:501-505.

46. Mårin P, Holmäng S, Jönsson L, et al. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obesity 1992;16:991-997.

47. Liu L, Merriam GR, Sherins RJ. Chronic sex steroid exposure increases mean plasma growth hormone concentration and pulse amplitude in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab 1987;64:651-6.

48. Yang S, Xu X, Björntorp P, Edén S. Additative effects of growth hormone and testosterone on lipolysis in adipocytes of hypophysectomized rats. J Endocrinol 1995;147:147-152.

CLAIMS

1. Use of growth hormone, preferably human growth hormone, or analogues thereof for the manufacturing of a medicament for treatment of The Metabolic Syndrome.

2. Use of growth hormone, preferably human growth hormone, or analogues thereof for the manufacturing of a medicament for prevention and treatment of non-insulin dependent diabetes mellitus.

3. Use according to claim 1 or 2 in which the medicament is for treatment of abdominal/visceral obesity.

4. Use according to any preceding claims in which the medicament is for increase of insulin sensitivity.

5. Use according to any preceding claims in which the medicament is for decrease of insulin resistance.

6. Use according to any preceding claims in which the medicament is for a treatment of at least six weeks, preferably more than six months and preferably more than nine months.

7. Use according to any preceding claims in which the medicament is for decrease of total body fat mass.

8. Use according to any preceding claims in which the medicament is for decrease of total body fat in human with abdominal/visceral obesity.

9. Use according to any preceding claims in which the medicament is for visceral adipose tissue area.

10. Use according to any preceding claims in which the medicament is for decrease of serum concentration of triglyceride.

11. Use according to any preceding claims in which the medicament is for decrease of diastolic blood pressure.

5

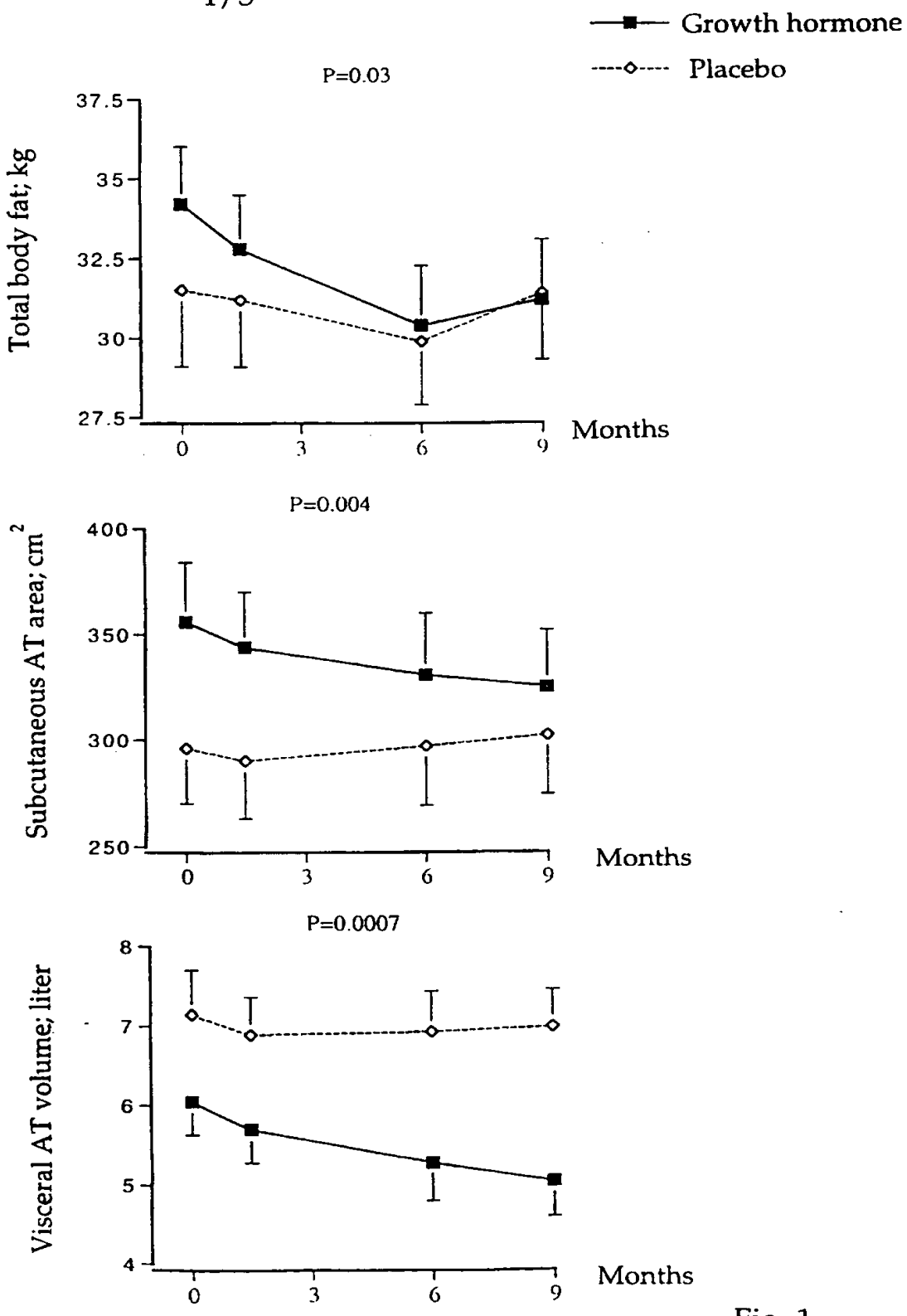
Growth hormone
Placebo
P=0.03
Total body fat; kg
37.5
35
32.5
30
27.5
0
3
6
9
Months
P=0.004
Subcutaneous AT area; cm 2
400
350
300
250
0
3
6
9
Months
P=0.0007
Visceral AT volume; liter
8
7
6
5
4
0
3
6
9
Months

Fig. 1

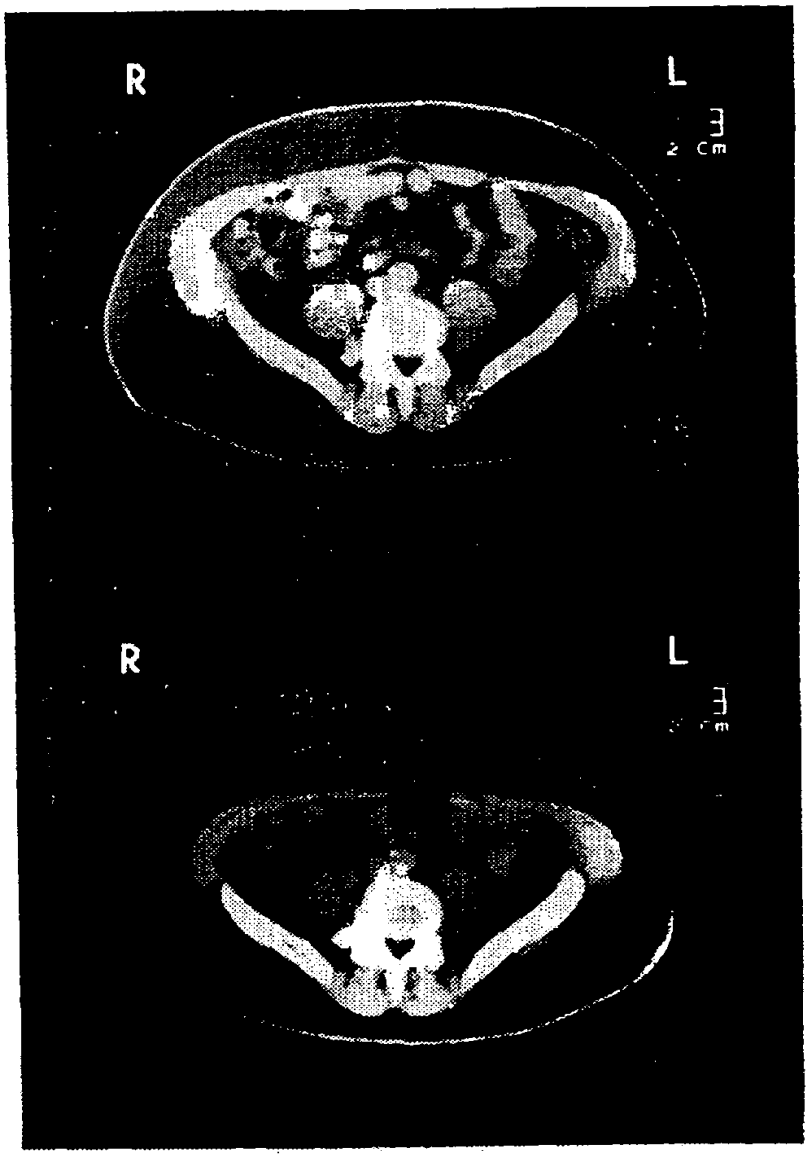

Fig. 2

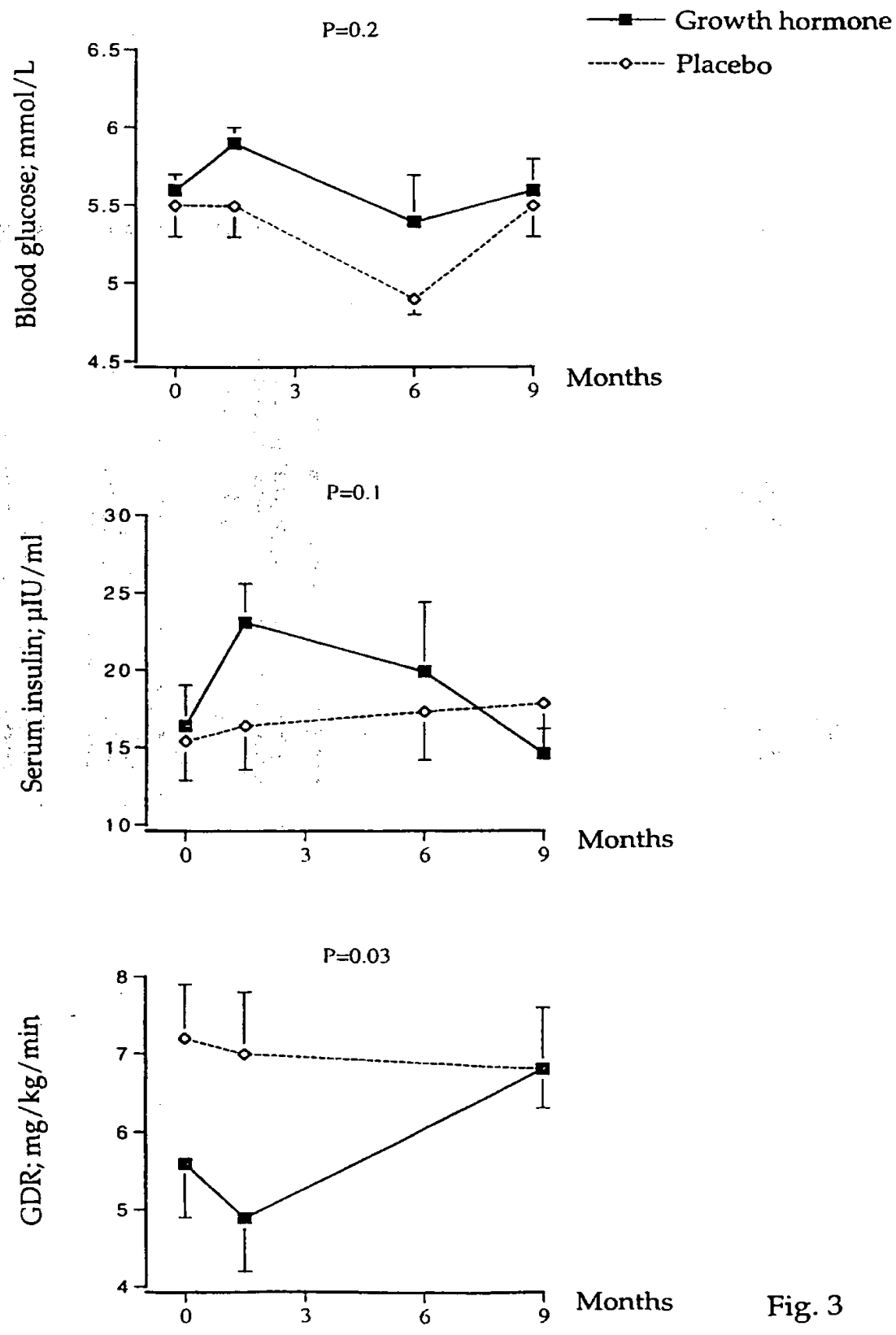
Growth hormone
Placebo
P=0.2
Blood glucose; mmol/L
6.5
6
5.5
5
4.5
0
3
6
9
Months
P=0.1
Serum insulin; µIU/ml
30
25
20
15
10
0
3
6
9
Months
P=0.03
GDR; mg/kg/min
8
7
6
5
4
0
3
6
9
Months

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00601

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 38/27
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, MEDLINE, EMBASE, WPIDS, IFIPAT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Journal of Clinical Endocrinology and Metabolism, Volume 82, No 8, 1997, Gudmundur Johannsson et al, "Growth Hormone Treatment of Abdominally Obese Men Reduces Abdominal Fat Mass, Improves Glucose and Lipoprotein Metabolism, and Reduces Diastolic Blood Pressure" page 727 --	1-11
P,X	Endocrinology and Metabolism, Volume 3, 1996, F Lee Hew et al, "Effects of Growth Hormone Deficiency and Therapy in Adults on Skeletal Muscle Glucose Metabolism, Lipid Profiles and Regional Body Composition" page 55 - page 60 --	1-11

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document blt published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 1997	29 -07- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00601

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Acta Paediatr Suppl, Volume 383, 1992, B-Å Bengtsson et al, "Effects of growth hormone on fat mass and fat distribution" page 62 - page 65	3-4,7-9
X	Horm Res, Volume 42, 1994, Jens O.L. Jorgensenet al, "Adult Growth Hormone Deficiency" page 235 - page 241	3-4,7-9
X	US 5268277 A (GUIDO GRANDI ET AL), 7 December 1993 (07.12.93)	3-4,7-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/00601

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5268277 A	07/12/93	DE 3879823 A	06/05/93
		EP 0306673 A,B	15/03/89
		SE 0306673 T3	
		ES 2053635 T	01/08/94
		JP 2000446 A	05/01/90
		JP 7004254 B	25/01/95